

REMARKS

I. STATUS OF THE CLAIMS:

Claims 68, 138, 142, and 145-192 are pending. New claims 187-192 have been added. Support for the new claims is found throughout the original specification as filed. Applicants respectfully submit that no new matter has been added by virtue of the present amendments.

II. REJECTION UNDER 35 U.S.C. § 102(b)

A. Rejection in view of GB 953,997.

In the Office Action, the Examiner rejected claims 68, 138, 142, and 145 on the grounds of being anticipated by GB 953,997 (‘the ‘997 reference’). The Examiner stated that “the instant claims read on the therapeutic use of the reference disclosed compound. The reference teaches a β -alanine compound ... and the corresponding therapeutic use of the compound. The reference teaches that the compound showed activity on neurological accident, specifically in a case of epilepsy.”

This rejection is respectfully traversed. Applicants respectfully submit that the ‘997 reference does not teach or suggest that β -alanine has any effects on epileptogenesis. Rather, the ‘997 reference describes that β -alanine has the following actions:

1. Action against sudden flashes;
2. Protection against redness and congestive phenomena caused by nicotinic acid, and its salified or esterified derivatives; and
3. Action against reactions of the allergic type
(see page 2, lines 84-115 of the ‘997 reference).

Applicants respectfully submit that the example in the ‘997 reference which “showed activity on neurological accident, specifically in a case of epilepsy” was an admixture of β -alanine and lysine nicotinate. Applicants respectfully submit that in this

example, the intent of the lysine nicotinate administration was to treat epilepsy and the intent of the β -alanine administration was to treat the side effects associated with the lysine nicotinate administration. This is evident at page 2, line 21 to page 3, line 12 of the '997 reference which states as follows:

"... β -alanine provides a very effective protection in man against vasomotor or general cutaneous incidents which are usually attributed to an excess of histaminemia ... produced in certain cases by the administration of nicotinic acid and pharmaceutically acceptable salified or esterified derivatives thereof and in particular lysine nicotinate, with which the β -alanine can thus be admixed so as to avoid their secondary effects..." (Emphasis added)

In view of the description in the '997 reference, one skilled in the art would recognize that the β -alanine/lysine nicotinate admixture utilized the lysine nicotinate with the intent to treat epilepsy and utilized β -alanine with the intent to treat the side effects of the lysine nicotinate. Therefore, this reference does not anticipate the present claims. This position is supported by Rapoport v. Dement, 254 F.3d 1053, 1059 (Fed. Cir. 2001). In Rapoport, the claim at issue stated in relevant part "[a] method for treatment of sleep apneas comprising of a therapeutically effective regimen ... [of buspirone] to a patient in need of such treatment" 254 F.3d at 1056. Rapoport argued that prior art anticipated the claim. The Board of Patent Appeals and Interferences found that although the prior art addressed treatment of a symptom of sleep apnea, the prior art did not address treatment of sleep apnea. *Id.* at 1060. The Court affirmed, reasoning in part that "there is no disclosure in [the prior art] of tests in which buspirone is administered to patients suffering from sleep apnea with the intent to cure the underlying condition." *Id.* at 1061. (Emphasis added) The Court noted that the prior art mentioned the possibility of administering buspirone to patients with sleep apnea, but explained that it was "for the purpose of treating anxiety in such patients, not *for the purpose of* treating the sleep apnea disorder itself[.]" *Id.* (Emphasis added). Thus, the Court rejected Rapoport's argument that the reason for administering buspirone to the patient was irrelevant. *Id.*

In view of Rapoport, Applicants respectfully submit that as the β -alanine in the '997 reference was administered to an epileptic patient without an intent to treat the

epilepsy, but rather to treat the side effects of lysine nicotinate, the reference does not anticipate the present claims.

Therefore, the Examiner is requested to remove the anticipation rejection of claims 68, 138, 142, and 145 over the '997 reference.

B. Rejection in view of U.S. Patent No. 4,375,477 to Bey et al.

In the Office Action, the Examiner rejected claims 68, 138, 142, and 145 on the grounds of being anticipated by Bey et al. The Examiner stated that “the instant claims read on the therapeutic use of the reference disclosed compound. The reference teaches a substituted β -alanine compound, see the structural formula I in col. 2, and the corresponding therapeutic use of the compound. The reference teaches that the compound is useful in the treatment of central nervous system disorders such as seizure disorders associated with epilepsy, see col. 3, lines 38-43.”

This rejection is respectfully traversed. Bey et al. describes at column 2 lines 14-24 that the “compounds of the invention” have a mandatory substitution of a monofluoromethyl or difluoromethyl at the two carbon linking group of Formula I. Applicants respectfully submit that the present claims do not encompass monofluoromethyl or difluoromethyl substitutions of the two carbon spacer unit of the present claims.

Applicants note that a trifluoromethyl compound (Compound E) was tested by Bey et al. in Example 13. However, Bey et al. stated that “in the case of Compound E, there was no protection against the seizures; indeed the seizures appeared to be potentiated.” (Emphasis added).

Accordingly, Applicants respectfully submit that the testing of Compound E in Example 13 does not anticipate the methods of treating a convulsive disorder as recited in

independent claims 138 and 142, as Example 13 reports that the trifluoromethyl compound is “inactive” at Table 1, column 18 of Bey et al.

Applicants further submit that Bey et al. does not anticipate the method of claim 68 as the reference does not report any testing for the inhibition of epileptogenesis, which is the process whereby normal brain is transformed into a state susceptible to seizures (see page 1, lines 22-26 of the specification). In support of this position, Applicants submit with this response copies of two papers from the text *Rational Polypharmacy*, Leppik, Ed. (ISBN:0-444-82455-3, Elsevier Science BV, 1996) by E. Lothman (pp. 3-7) and D. Lowenstein (pp. 45-60.) Lothman defines *ictogenesis* as processes involved in initiation, elaboration and extension of seizures. Epileptogenesis, on the other hand, is a different phenomenon, and is defined as long-term, progressive changes in neural networks that eventually provoke spontaneous and recurring seizures. Epileptogenesis involves processes that take place before the first seizure occurs, rendering the epileptic brain susceptible to spontaneous recurrent seizures, which processes serve to intensify seizures and make them more refractory to therapy. Epileptogenesis also includes processes during a seizure that lead to its full expression and the sequence of events enacted after a seizure is over that influence the brain for a finite period of time, or indefinitely.

Applying these definitions, Applicants respectfully submit that Bey et al. does not and cannot anticipate the method of inhibiting epileptogenesis as recited in claim 68. In column 19 of Bey et al., it is stated that Compounds A-E were tested “to determine the extent of protection against mercapto propionic acid induced seizures.” Clearly, this description in Bey et al. is directed to the use of the compounds therein to suppress or inhibit seizures in progress and the compounds are not administered to inhibit epileptogenesis, as seizures have already been chemically induced in these experiments prior to the administration of the test compounds. Therefore, Bey et al. cannot anticipate a method of inhibiting epileptogenesis, since in the experiments a) seizures have already been induced, and b) the intent of Bey et al. is to treat seizures and not to inhibit epileptogenesis.

In view of the arguments presented above, the Examiner is requested to remove the anticipation rejection of claims 68, 138, 142, and 145 over Bey et al.

**IV. NONSTATUTORY OBVIOUSNESS-TYPE DOUBLE
PATENTING REJECTION**

In the Office Action, the Examiner provisionally rejected claims 68, 138, 142 and 145-186 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 60-99 of copending Application No. 10/272,249.

Further, the Examiner provisionally rejected claims 68, 138, 142, 145 and 151-162 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 60-99 of copending Application No. 11/099,232.

In response, Applicants respectfully submit that upon indication that the present claims are otherwise allowable, the filing of terminal disclaimers to overcome the Examiner's nonstatutory obviousness-type double patenting rejections will be considered.

V. **CONCLUSION**

This Response is being submitted together with a petition for a two-month extension of time under 37 C.F.R. § 1.136(a) from July 20, 2006 to September 20, 2006. A check in the amount of \$ 225.00 is enclosed herewith to cover the fee due under 37 C.F.R. § 1.17(a)(2). It is believed that no other fees are due. If, however, it is determined that any additional fees are due or that any fee has been overpaid, the Commissioner for Patents is hereby authorized to charge said fee or credit any overpayment to Deposit Account No. 50-0552.

Respectfully submitted,

DAVIDSON, DAVIDSON & KAPPEL, LLC

By: _____

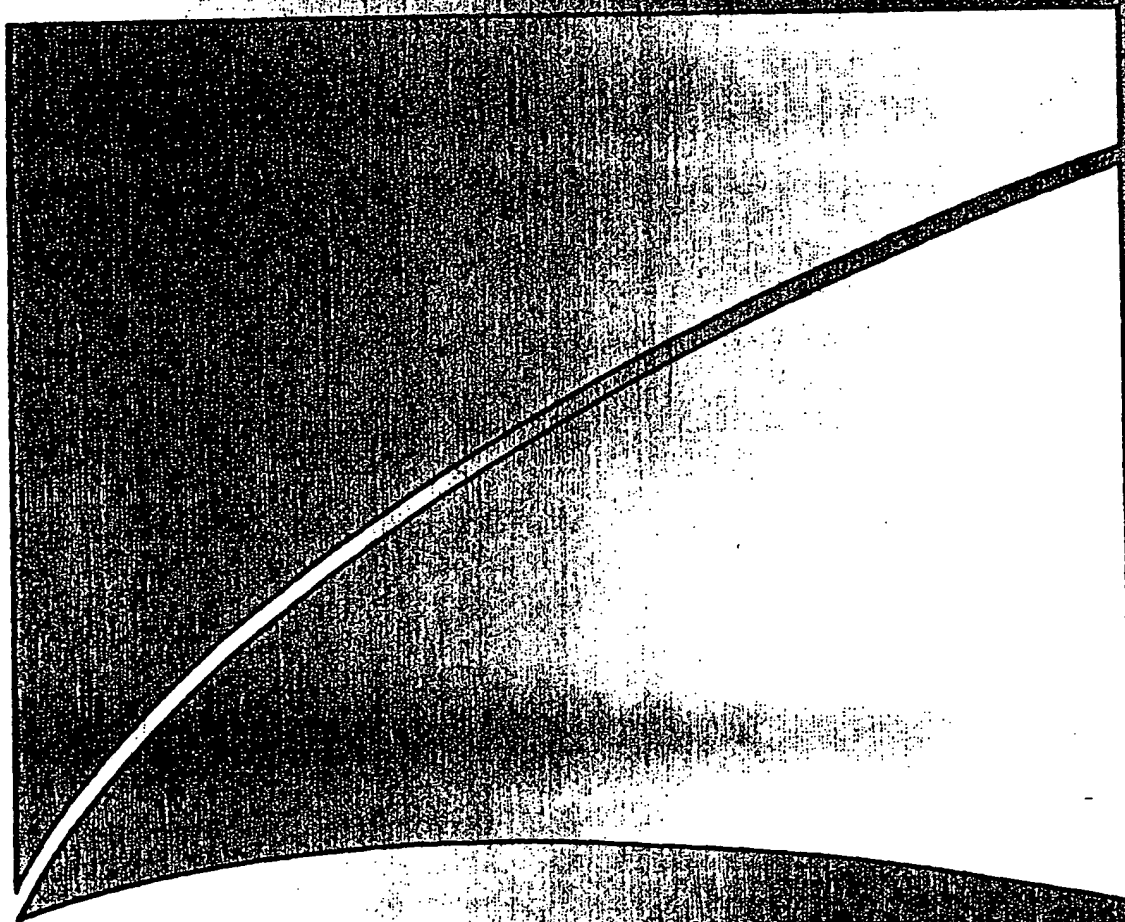

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RATIONAL POLYPHARMACY

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Neurobiology as a basis for rational polypharmacy Section Overview for Rational Polypharmacy Conference

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During the past two decades several concepts have evolved to the point of becoming guiding principles in the clinical use of pharmaceutical treatments for epilepsy and seizures. Current practice recognizes the need for accurate diagnosis (establishing the presence of seizures as opposed to other types of 'spells' and the proper classification of them with respect to seizure types and epileptic syndromes) and the use of agents appropriate for the particular diagnosis. These principles emerged from a wealth of advances in clinical epileptology and clinical pharmacology of antiepileptic drugs. Furthermore, the principle of monotherapy emerged, driven by clinical experience in the 1970s. At this time the profiles of clinical efficacy of antiepileptic drugs had not been elucidated nor had the classification schemes for seizures and epileptic syndromes been formulated. Accordingly, an appropriate drug, in terms of seizure suppression, was often not matched with the clinical condition. Consequently, patients were frequently treated with polypharmacy. This approach, combined with the lack of readily available blood level monitoring of antiepileptic drugs, led to frequent toxicity. The move to monotherapy grew out

of this context and, in combination with the advances mentioned above, led to successful treatments for many patients. However, up to a third of patients today do not achieve adequate control of their seizures with medications. In these individuals, the standard approach is to try two or more medications, each used as monotherapy, and then resort to multiple concurrent medications. Yet, guidelines for such polypharmacy have not been established.

In parallel with the progress in the clinical realm cited above, tremendous strides were made in our knowledge of the basic neurobiology behind seizures and epilepsy. Advances in this realm include: the development and refinement of animal models that are counterparts of specific seizure types in humans (including acute seizure models and chronic epilepsy models); the development and refinement of animal models and test systems useful in identifying new anti-epileptic agents; identification of new agents for treating epilepsy and seizures; a broadened understanding of the mechanisms of action exerted by many of the anti-epileptic drugs in current use; identification of the circuits involved in various types of seizures; elucidation of fundamental alterations that distinguish the normal brain from the epileptic brain and exploration of how the various stages of maturation (neonate, youth, adult, senescence) impact on seizures and epileptogenesis. Such information will be useful in developing ideas and guidelines for polypharmacy.

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note
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time-line

From considerations of the basic science of seizures and epilepsy, a broad domain emerges in which the term polypharmacy can be applied. Conceptually, a variety of phenomena are subsumed in the term epileptogenesis (Fig. 1). These include: processes in the chronically epileptic brain which make it susceptible to spontaneous recurrent seizures; processes during a seizure that lead to its full expression; the sequence of events enacted after a seizure is over that influence the brain for a finite period of time or indefinitely; and interactions between the processes just mentioned with the events that occur during maturation and involution of the brain. Indeed, there is a multiplicity of points on the spectrum of pathophysiology at which one could potentially intervene therapeutically, i.e. at which polypharmacy would be appropriate. Moreover, it should also be emphasized that for each of the general categories mentioned in the spectrum of pathophysiology, multiple processes occur. Thus, even if one restricted treatment to a single 'site' on the spectrum of pathophysiology, there is the possibility of polypharmacy. At present the scope of such proposed polypharmacy

is speculative; for many of the types of processes identified, drugs have not yet been developed and our knowledge of the basic science is rudimentary. However, the considerations just raised provide a theoretical framework for a broader, and more effective, treatment for epilepsy, including the cases that remain refractory to current therapeutics.

In fact, at least one of the theoretic principles raised above has become incorporated into current thinking. Based on the discussion above, one can distinguish *ictogenesis* (processes involved in initiation, elaboration, and extension [in time and space] of seizures) from *epileptogenesis* (processes involved in augmented propensity for spontaneous seizures or in the progression in severity of seizures or their resistance to medical therapy). In this regard then, an *anti-ictal drug* (i.e. one suppressing seizure expression) would be distinguished from an *anti-epileptogenic drug* (i.e. one opposing one or more aspect of epileptogenesis). The proposed term anti-ictal drug would replace the term anti-epileptic drug in current usage. While displacing an established term that many are familiar with and comfortable,

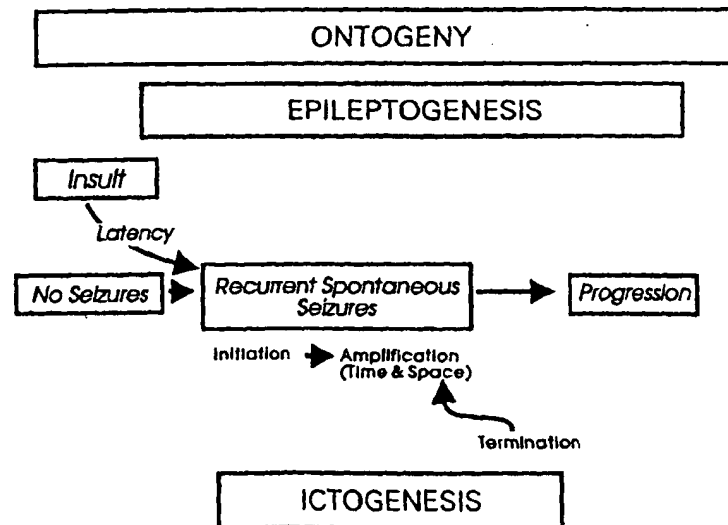


Fig. 1. Spectrum of pathophysiology for seizures and epilepsy. Each individual seizure, whether an isolated, 'provoked' seizure or a seizure as part of epilepsy, can be separated into phases in which seizure initiation occurs and in which seizure elaboration, involving spread in time and space, occurs. In addition, certain processes serve to terminate a seizure; when these fail, status epilepticus ensues. The term *ictogenesis* is applied to the just-mentioned processes in aggregate. The term *epileptogenesis* subsumes ictogenesis, but also involves processes that take place before the first seizure occurs to render the epileptic brain susceptible to spontaneous recurrent seizures, processes that serve to intensify seizures and to make them more refractory to therapy (progression).

the new term avoids ambiguities inherent in the terminology of anti-epileptic versus anti-epileptogenic drug, at least in the context of the current discussion.

An anti-epileptogenic drug may also exert an anti-ictal effect, but this is not necessarily so. In fact, in view of certain current hypotheses about the pathophysiology of chronic epilepsy, one could argue that at least certain agents effective against epileptogenesis would be expected not to possess anti-ictal effects (e.g. anti-oxidants, compounds interacting with neurotrophic factors, and, possibly, neuroprotective drugs). Likewise, an anti-ictal agent will not necessarily possess anti-epileptogenic effects. These predictions are born out by work with the kindling model which has identified some compounds that retard kindling (and therefore can be considered anti-epileptogenic) but are not anti-ictal, some compounds that exert both an anti-ictal effect and an anti-epileptogenic effect, and some compounds that exert an anti-ictal effect but do not retard kindling. The work with the kindling model also predicts that clinical use of specific anti-epileptogenic agents is a reasonable and viable goal.

Two fundamental dichotomies in the current ILAE Classification of seizure types are noteworthy. The first dichotomy involves the distinction between seizures that involve paroxysmal discharges throughout all, or most of, the brain from the beginning of a seizure, so-called primary generalized seizures, and seizures that remain restricted to certain regions of the brain, so-called partial seizures (Fig. 2). Seizures that begin as partial seizures may show secondary generalization. This dichotomy stresses the importance of networks in seizures and how they interact to sustain, amplify and propagate seizures. The second dichotomy stressed here is the distinction between primary generalized absence seizures and generalized tonic-clonic seizures. While the terminology derives from clinical phenomena during the seizures, there is also a strict separation of the electrographic discharges that account for these two types of seizures. Furthermore, the behaviors of neurons during the two types of seizures are very different and rely on fundamentally different types of abnormalities in terms of cellular and synaptic events.

Thus, seizures are neuronal network phenomena and the networks are comprised of heterogeneous

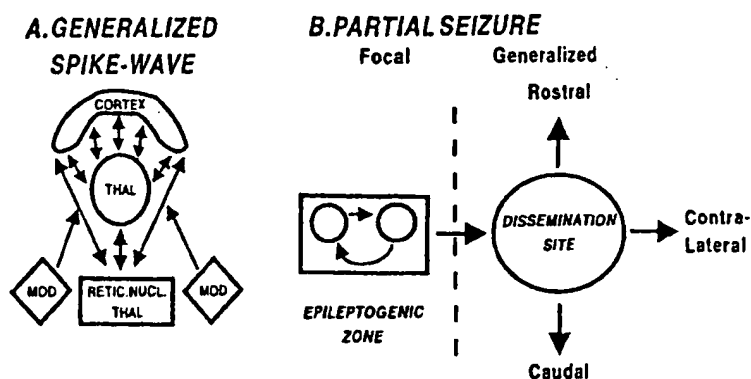


Fig. 2. Functional anatomy and ictogenesis. Seizures can be separated into those that are generalized or those that are partial. Moreover, there is a dichotomy of generalized seizures, involving tonic-clonic convulsions and absence paroxysms with spike-wave discharges. Spike-wave seizures involve a complex interaction between the thalamus and cortex, as well as components in the thalamus, including the reticular nucleus and specific relay nuclei; in addition, intrinsic membrane properties of thalamic cells (see Chapter 2). Thus, the paroxysms involve a widely distributed system and, in fact, incorporate many aspects of normal brain functioning underlying sleep rhythms. Ascending modulatory systems (MOD), such as aminergic and cholinergic tracts, from the brainstem can also interact with the thalamocortical rhythm generating system. In contrast, partial seizures (B) involve seizures that begin in a restricted region. While the concept of a highly localized 'epileptic focus' has been discussed for some time, current notions are of a wider epileptogenic zone that is responsible for seizure generation. Within that zone, there may be more than one site at which seizures begin and two such sites may interact in a re-inforcing fashion to provide positive feedback in the elaboration of a focal (partial) seizure. At times the seizure processes may spread in space from the epileptogenic zone to certain dissemination sites (e.g. piriform cortex, 'area tempestas') and from there become generalized with propagation to the contralateral side, to rostral brain areas and to caudal central nervous system areas.

elements that play different roles in the expression of seizures. In theory, agents may interact with different potencies at different sites in the networks. For instance, one drug may be more effective in suppressing seizure initiation at a primary epileptic focus while another drug may be more effective at an anatomical site responsible seizure generalization. Moreover, neuroscientists are uncovering marked heterogeneities between various anatomical sites in terms of subunit composition of excitatory (glutamatergic) and inhibitory (GABAergic) amino acid receptors, as well as local circuits (feedback inhibition, feedforward inhibition, feedforward excitation). Therefore, a drug with a particular cellular action may exert different properties at different sites, e.g. thalamic versus neocortical versus limbic (amygdala, hippocampus). Consequently, there is a rich potential for multiple sites of actions when one considers neuronal circuits linking various brain regions, the wide variety of types of neurons within particular brain regions and how they are linked in local circuits. Given this variety, there is potential for rational polypharmacy on the basis of molecular, cellular and network actions of different therapeutic agents.

With the explosion in information about basic neurobiology we have learned much about how the brain grows and develops and how the immature brain differs from the mature brain. An evolving field is how the senescent brain differs from the 'normal' adult brain. Powerful tools, including molecular biology, genetic rodent models, and transgenic mice, are providing inroads into understanding how genetic factors are involved in shaping normal, and abnormal, operations of the central nervous system. While this field is truly in its infancy in terms of providing a core of basic information, it holds great promise over the coming years. These dimensions also provide new aspects for polypharmacy. One can easily hypothesize that a particular gene is expressed only at a particular age and this expression is solely responsible for or critically interacts with pathophysiological processes common to all ages in shaping the phenomenology of age-specific epilepsy syndromes. One could also postulate a like involvement of a particular gene, expressed as a result of heredity, in human genetic epilepsies.

The foregoing discussion provides a framework

for a number of the following papers. Lothman (Chapter 2) covers the pathophysiology of seizure expression in terms of basic cellular and synaptic mechanisms. Löscher (Chapter 3) covers the topic of seizure propagation in terms of functional anatomy of seizure propagation. Lowenstein (Chapter 4) covers the topic of epileptogenesis, as distinguished from seizure expression, highlighting potential areas for therapeutic intervention separate from anti-ictal therapy. Meldrum (Chapter 6) reviews known mechanism of action of current anti-ictal agents, covering concepts of seizure threshold and cellular and molecular sites of action. Wolf (Chapter 11) addresses the interface between the various epilepsy syndromes that have been identified by epileptologists and the potential this affords for new modes of therapy.

The information provided in these articles was used as a point of departure for in-depth workshops held at the conference. A major objective of the workshops was to identify areas of agreement and of disagreement, as well as what is known and what is not known about the subject of rational polypharmacy with the aim of identifying what type of work should be done next, what questions should be asked, and how these questions should be studied. On the basis of work done at the conference, certain consensus statements were reached that have been incorporated into the chapters mentioned above. In addition, the workshops dealt with the issue of how best to integrate animal work with clinical work. The conclusion was that animal work would clearly be helpful but, at the present time, our experimental methodology was not well enough developed in the

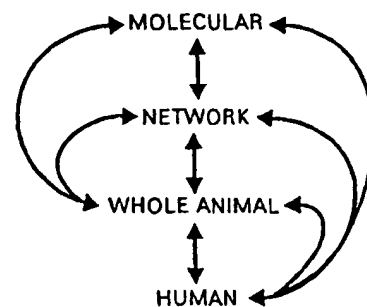


Fig. 3. Proposed approach for systematic development of rational polypharmacy, emphasizing iterative interaction between basic science and clinical studies and various "levels" of basic neuroscience.

realm of polypharmacy to copy the paradigm of new single anti-epileptic drugs, namely initial preclinical work followed by clinical studies. Rather, recommendations were that the *overall* approach to systematically developing the field of rational polypharmacy should be iterative, with interactions between clinical and basic science and at the various levels of basic science (Fig. 3). Moreover, recommendations stemming from workshops at the conference emphasized the needs to: establish a better foundation for rational polypharmacy by strengthening our understanding of the fundamental pathophysiology of seizure expression and epileptogenesis; and devise,

test, and refine animal models for studying rational polypharmacy. Activities directed at the last recommendation are considered in Chapter 5 by Löscher and Wauquier. An overarching theoretical scheme for the practice and study of rational polypharmacy is provided in the chapter by Macdonald (Chapter 7), and is interfaced with a survey of mechanisms of action of current antiepileptic drugs.

Acknowledgements

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Recent advances related to basic mechanisms of epileptogenesis

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Abstract

A variety of clinical observations suggest that certain forms of epilepsy are due to long-term, progressive changes in neural networks that eventually provoke spontaneous and recurring seizures. This process of network transformation, known as epileptogenesis, is a potentially important therapeutic target and also serves as an extremely interesting model of central nervous system plasticity. This article reviews some of the significant, recent advances in our understanding of mechanisms underlying epileptogenesis in different forms of epilepsy. The most substantial progress has been made in work related to temporal lobe epilepsy (TLE), where the biochemical, electrophysiological and anatomical changes in the hippocampus have been intensively studied. This has led to a number of cogent and testable hypotheses, including the concept that dentate granule cell hyperexcitability in TLE is due to a selective loss of hilar neurons that renders inhibitory cells 'dormant.' Studies of other forms of focal epilepsy suggest that a seizure focus may develop as a result of axonal reorganization or immune-mediated effects on membrane channels. Epileptogenesis in generalized epilepsies remains poorly understood, although recent work using models of absence epilepsy point to the critical role of GABA_B or T-type calcium channels in the thalamus. Also, new transgenic mouse lines with epilepsy phenotypes have introduced candidate genes, such as those encoding the serotonin 5-HT_{2C} receptor or the alpha subunit of calcium/calmodulin kinase II, that may be responsible for epileptogenesis. Finally, a large amount of investigation has focused on seizure-induced gene expression and it is now clear that seizures can cause a cascade of changes in the expression of gene products that are likely to play a role in network plasticity. Progress in developing 'anti-epileptogenic' therapies will require further advances in understanding the mechanistic roles of these various biochemical and anatomical changes in the transformation of normal to hyperexcitable neural networks.

Keywords: Seizure; Epileptogenesis; Temporal lobe; Dentate gyrus; Trauma; Rasmussen's syndrome; Absence; Transgenic; Neurotrophin; Growth factor; Ion channel; Rational polypharmacy

1. Introduction

Epileptogenesis refers to the process in which a normal neural network transforms into a hyperex-

citable network with an abnormal seizure threshold. In its broadest sense, the concept of epileptogenesis has been applied to any network transition that culminates in an epileptiform discharge. However, in this chapter, the term epileptogenesis will be used in a somewhat more restricted sense, i.e. the longer-term, permanent changes in neural networks that provoke spontaneous and recurring seizures [64].

The clinical importance of the process of epileptogenesis is best illustrated by the abundant evidence showing that there is often a considerable temporal

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¹ Presented at the International Conference for the Definition and Debate of Rational Polypharmacy, Durango, Colorado, September 1994.

delay between an initial central nervous system (CNS) insult and the eventual development of epilepsy. This observation was documented at least as early as the 15th century, when Berengarius da Carpi and Duretus each described patients who developed epilepsy months to years after head injury [114]. Many contemporary studies of large series of patients have shown that the time delay between the initiating event and epilepsy can span from weeks to many years. For example, the risk of developing epilepsy following severe head injury remains elevated for at least 10 years or longer [2,94]. Likewise, it is well recognized that various 'idiopathic' epilepsies appear at a particular developmental age. Absence epilepsy tends to appear during childhood and juvenile myoclonic epilepsy presents during adolescence. *These observations emphasize the importance of looking at the underlying mechanisms of epilepsy as dynamic processes.*

This chapter will explore some of the leading hypotheses that have been put forward to explain epileptogenesis in different types of epilepsy. The majority of the material will be devoted to advances in our understanding of the cause of temporal lobe epilepsy, since the most significant recent strides have been made in this area. However, epileptogenesis in generalized epilepsy will also be discussed, particularly the recent studies suggesting that altered function of the GABA_B and T-type calcium channel systems are seen in models of absence epilepsy. Finally, an overview is provided of the recent work showing that various forms of epileptogenesis are associated with both acute and long-term changes in CNS gene expression. Such changes in gene expression are likely to play a role in the relatively long-term changes in network structure or function that are hallmarks of various forms of epilepsy.

2. Epileptogenesis of focal epilepsy

2.1. Temporal lobe epilepsy

2.1.1. Background

Advances in the understanding of the pathophysiologic basis of temporal lobe epilepsy (TLE) have been driven by two features of this particular form of focal epilepsy. First, the focus of seizure activity

within the temporal lobe is frequently localized to the hippocampus, which, relative to other regions in the forebrain, is an unusually well-defined anatomic structure that is very amenable experimentally to structural and functional analyses. Second, since the 1940s many patients with TLE have been successfully treated by the surgical removal of the anterior portion of the temporal lobe, providing access to the structures responsible for either the source or propagation of seizures in TLE. As recently emphasized by Sloviter [104], the availability of pathological specimens from patients with TLE has provided important anatomical clues regarding the underlying functional defect in this disorder. In particular, many patients have a relatively selective loss of specific subpopulations of neurons in the hippocampus, especially within the hilus of the dentate gyrus [25,102,104]. There is also often abnormal dispersion of the dentate granule cell layer [48] and 'sprouting' of mossy fibers (the axons of the dentate granule cells) into the inner molecular layer of the dentate gyrus [3,25,50,110].

Although these clinical observations strongly support the notion that hippocampal dysfunction is an integral component of the pathophysiology seen in many TLE patients, the relative importance of the hippocampus in TLE compared to other temporal lobe regions remains a matter of heated debate. For example, in the past few years, substantial attention has been drawn to the potential roles of the parahippocampal gyrus, entorhinal cortex and amygdala as important structures involved in TLE [5,15,28,35]. Currently, however, the relationships between structure and function in these other limbic regions relevant to epilepsy are relatively poorly understood compared to what is known about the hippocampus.

2.1.2. Animal models

A variety of animal models have been developed over the past 20–25 years which share important characteristics with human TLE. Kindling, discovered by Goddard in 1967 [43], is considered one of the better models of epileptogenesis because the creation of a permanent epileptic state is dependent on a series of subconvulsant stimuli delivered over a relatively long period of time (see article by Löscher in this issue). The pathological features in the hippocampus induced by kindling include hilar cell loss

and mossy fiber sprouting [13,108,111]. These changes are rather subtle compared with what is observed in human tissue. Sloviter's model of prolonged stimulation of the perforant path in rats replicates the pathological lesion of TLE much more closely [106]. Similar results have been obtained by Lothman and colleagues with a model of chronic temporal lobe epilepsy that follows limbic system status epilepticus induced by focal hippocampal stimulation [6,8,63]. Among many techniques using chemoconvulsants, the kainic acid and pilocarpine models have been especially popular since they also replicate TLE pathology [7,62,75,76,84,105]. However, the kainate and pilocarpine models suffer from variability in the extent and severity of pathological changes, especially when the compounds are used systemically.

It is important to note that the majority of work in these various animals models has focused primarily on acute and chronic anatomical changes, as well as the responses of neuronal populations to experimentally induced electrophysiological or pharmacological stimuli. In contrast, relatively little is known about the spontaneously occurring seizures that are frequently described in these models. Some investigators are beginning to study this by setting up chronic recording systems that allow on-line video and electrophysiological monitoring of animals over long periods (E. Lothman and E. Bertram, personal communication). These studies should provide critical information regarding the validity of these approaches as models of TLE.

2.1.3. Selective loss of hilar neurons

One of the most compelling hypotheses to emerge over the past decade regarding the cause of TLE suggests that a selective loss of hilar neurons leads to abnormal disinhibition of dentate granule cells, the principal cell group projecting out of the dentate gyrus. Hilar neurons are comprised of numerous subtypes of cells that have various anatomical and electrophysiological characteristics. Two of these subtypes, the mossy cells and basket cells, are thought to participate in an inhibitory local circuit that serves to regulate the excitability of neighboring dentate granule cells (see Fig. 1A). In 1983, Sloviter showed that the highly selective loss of dentate hilar neurons caused by prolonged perforant path stimulation was

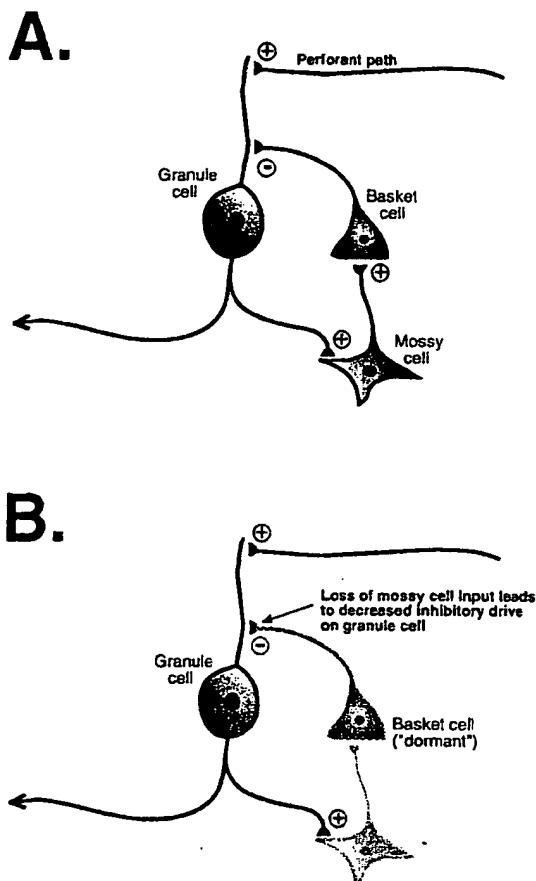


Fig. 1. (A) Schematic of a simple neuronal network in the dentate gyrus. A dentate granule cell receives afferent input from the entorhinal cortex via the perforant pathway and the granule cell's main projection is to CA3 pyramidal cells. A recurrent inhibitory network is depicted between the granule cell, hilar mossy cell and basket cell. (B) Network disinhibition due to selective vulnerability of the hilar mossy cell. The loss of the mossy cell interrupts recurrent inhibition. As suggested by Sloviter [102], the basket cell is intact but 'dormant,' and could potentially regain function if provided with the appropriate stimulus. Note that this schematic is highly simplified and evidence for the existence of the normal and dysfunctional network as shown remains incomplete.

associated with marked disinhibition of the dentate granule cells, as measured by responses to paired-pulse stimulation [101]. More recently, we have found that the same pattern of selective loss of hilar neurons can be caused by a single, focal, percussive force against the cortical surface overlying the hippocampus (Fig. 2) [70]. Importantly, this pattern of

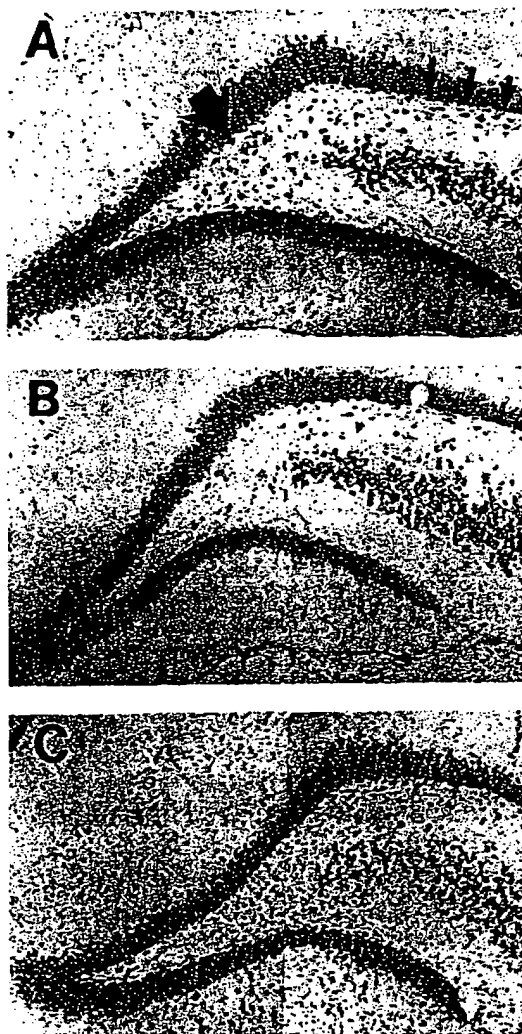


Fig. 2. Selective vulnerability of hilar neurons 7 days following traumatic fluid percussion injury. (A) In a normal dentate gyrus, there are many intact neurons within the hilus as evidenced by the large, pleiomorphic, stained cell bodies within the deep hilus (large arrow) and immediately below the superior dentate granule cell layer (small arrows). Following low (B) and high (C) forces of percussion injury at the cortical surface, there is a highly selective loss of hilar neurons that correlates with the amount of impact. The very small, basophilic profiles in the hilus after high injury are glial elements related to the injury-induced gliosis. Magnification = 67 \times . For details, see Ref. [70].

injury is also associated with relative disinhibition of dentate granule cells. These observations thus offer a potential mechanistic connection for the association

of mechanical head injury or early prolonged seizures in humans and certain forms of focal epilepsies.

How does selective neuronal injury due to an acute process such as head injury or status epilepticus relate to epileptogenesis? One idea, suggested by Sloviter and depicted in Fig. 1B, is that an initial insult leads to a relatively restricted region of interneuron loss and focal, subclinical dentate granule cell hyperexcitability [102,104]. In a manner analogous to the perforant path stimulation model, this abnormal activity causes interneuron injury in a growing region of the hilus over time. When a critical number of interneurons are lost, the dentate gyrus becomes sufficiently disinhibited to become either a source of spontaneous, clinical seizures, or an amplifier of abnormal discharges emanating from other regions.

These concepts emphasize the potential importance of understanding the mechanisms of selective vulnerability of hilar neurons in order to develop methods for preventing epileptogenesis. The main afferents to the mossy cells are thought to be glutamatergic [1,16] and mossy cells express glutamate receptors [61,96], suggesting that the cell loss may be related to excitotoxicity. Nonetheless, the reasons for the marked, *selective* sensitivity of these cells to various insults remain unknown. It has been suggested that hilar interneurons have an impaired ability to buffer glutamate-mediated calcium toxicity, perhaps due to a lack of expression of certain calcium-binding proteins such as calbindin-D28K [107]. Important evidence supporting this concept was provided by Scharfman and Schwartzkroin, who demonstrated that excitation-induced injury of hilar interneurons could be prevented by intracellular injection of a calcium chelator [97].

The survival of GABAergic inhibitory neurons in the disinhibited dentate gyrus provides another potential anti-epileptogenic strategy. The 'dormant basket cell hypothesis,' proposed by Sloviter in 1991 [102], states that the remaining GABAergic basket cells, although quiescent due to the loss of excitatory mossy cell input, are still potentially functional if activated through an alternate mechanism. Evidence supporting this concept has come from studies looking at a similar network in the CA1 pyramidal layer [6,102]. Pharmacological agents that could selectively reactivate the 'dormant' GABAergic basket

cells would provide a means of renewing control of the activity of dentate granule cells. This, in turn, would prevent the ongoing seizure-induced hilar neuron injury that is proposed to underlie the development of a permanent, seizure-prone network in TLE.

2.1.4. Axonal reorganization

Another prominent anatomical change observed in the hippocampus both from patients with TLE and animal models is sprouting of dentate granule cell mossy fibers into the inner molecular layer of the dentate gyrus [50,110–112]. The projection sites of mossy fibers can be easily identified using the Timm's stain, a histochemical method that highlights the unusually high concentration of zinc found in mossy fiber terminals. Early studies provided evidence that at least some of the aberrantly sprouted mossy fiber terminals make synaptic contacts with dentate granule cell dendrites [34].

In 1985, Tauck and Nadler [112] suggested that the development of mossy fiber sprouting following kainate-induced seizures was associated with dentate granule cell hyperexcitability, as measured by responses to paired-pulse stimulation via the perforant path. These results were proposed to support the anatomical evidence that sprouting created recurrent excitatory networks, as depicted in Fig. 3. This concept was further advanced by Sutula et al., who documented mossy fiber sprouting in association with the development of kindling [111]. However, Sloviter showed that dentate granule hyperexcitability developed immediately (i.e. within days) after kainate seizures, well before the appearance of mossy fiber sprouting [103]. Furthermore, there was a progressive increase in dentate granule cell inhibition, measured by paired-pulse stimulation, in the weeks to months following the initial injury. We have observed the same pattern in animals with pilocarpine-induced sprouting (unpublished data). Dudek and colleagues [21] have suggested mossy fiber sprouting may cause a combination of abnormal hyperexcitability and inhibition in the dentate gyrus. Using *in vitro* analysis of hippocampal slices derived from sprouted versus control animals, they found that under normal recording conditions the dentate granule cells of sprouted animals were relatively inhibited. However, when the slices were treated with the

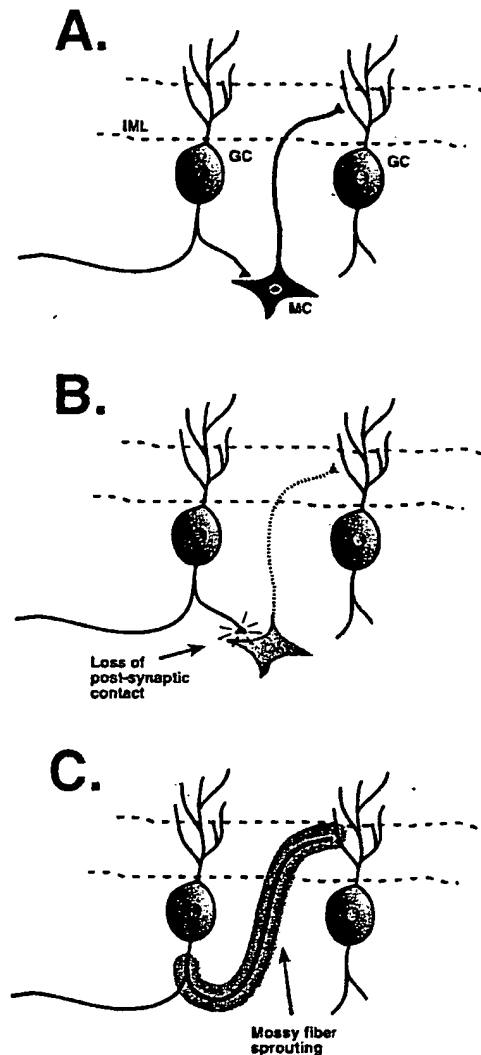


Fig. 3. Dentate granule cell axon sprouting following loss of mossy cells. (A) A part of a simple network is shown. Note that the granule cell normally has collateral projections to mossy cells. IML—inner molecular layer. GC—granule cell. MC—mossy cell. (B) If the mossy cell is injured, the granule cell loses one of its normal post-synaptic contacts. (C) This axon terminal is hypothesized to reorient itself, 'sprout' into the inner molecular layer and establish a new synaptic contact with a dendrite of neighboring granule cells. The new contact is presumably made on a post-synaptic site made available through the loss of mossy cells or other neurons that project to the inner molecular layer. As in Fig. 1, this schematic is highly simplified and does not show the many other forms of network reorganization that are presumed to exist following dentate gyrus injury.

GABA antagonist bicuculline, there was an 'unmasking' of underlying hyperexcitability in a subset of the sprouted slices, but not those derived from normal animals.

Taken together, the observations to date have shown that at least some sprouted mossy fibers make direct connections on dentate granule cell dendrites and this is an intuitively appealing mechanism whereby dentate granule cell hyperexcitability may develop during epileptogenesis. However, it is important to note that a direct, causal link between these anatomical and functional changes has not been established. Nonetheless, it seems reasonable to propose that the reorganization is altering the normal physiology of the dentate gyrus in some way, modifying either excitation, inhibition, or both.

It must also be emphasized that relatively little is known about other systems that may be reorganizing in addition to the dentate granule cell projections. For example, we have found that kainate- and pilocarpine-induced seizures can lead to a change in the pattern of cholinergic fibers in the supragranular layer of the dentate gyrus [66]. Babb and colleagues have also shown that GABAergic neurons also sprout under similar circumstances [24]. Thus, there are probably many forms of axonal reorganization in human and experimental models of epilepsy, and these need to be fully investigated in order to dissect apart the roles these play, if any, in epileptogenesis.

2.2. Other focal epilepsies

2.2.1. Epileptogenesis following traumatic cortical injury

As noted at the beginning of this article, some of the most compelling evidence for the concept of chronic epileptogenesis derives from observations of patients with acquired epilepsy whose seizures develop months or years following injury. The incidence of epilepsy following severe head injury, i.e. involving direct cortical injury, is 25–50% [14,94]. The epilepsy may develop immediately, or patients may have initial seizures followed by a 'silent period' and then the re-emergence of seizures [14]. A similar temporal pattern can also be seen in some patients who develop epilepsy following stroke or CNS infections.

With regard to the potential mechanisms underlying these conditions, it is now well established that traumatic brain injury can lead to a broad cascade of pathophysiological events, including changes in cerebral blood flow, breakdown of the blood-brain barrier, hemorrhage, infarction and axonal swelling, with secondary biochemical consequences including excitotoxicity and free radical injury [120]. However, remarkably few studies have directly examined the potential relationships between traumatic, cortical injury and alterations in network excitability. Work in the 1960s demonstrated that chronic isolation of regions of neocortex (e.g. by undercutting the cortex) could lead to abnormal, persistent excitability within the isolated region [30,98]. More recently, Prince and colleagues [46,86] have used *in vitro* slice preparations from partially isolated cortex and determined that (1) hyperexcitability develops with a latency of 1 to 2 weeks following injury, (2) layer V appears to be the source of interictal discharges and (3) the changes in excitability are not explained by disinhibition alone. Importantly, this group has subsequently shown that these electrophysiological changes occur in association with axonal sprouting of layer V neurons in the same cortical region and over a similar time period [95]. These observations represent a potential major step forward in understanding the mechanisms of epileptogenesis following certain forms of focal injury involving extratemporal cortex.

2.2.2. Rasmussen's encephalitis

A very recent study concerning Rasmussen's encephalitis provides yet another potential mechanism for focal epileptogenesis [93]. This epileptic syndrome, which affects children during the first decade of life, is characterized by the progressive development of intractable seizures, hemiparesis and dementia, in association with inflammatory changes of the cerebral cortex. While in the process of generating antibodies against glutamate receptor subunits. Rogers and colleagues found that one of the rabbits used for production of anti-GluR3 antibodies developed a seizure disorder and CNS pathology analogous to that observed in Rasmussen's encephalitis. Reasoning that patients with the disease might have similar autoantibodies, these investigators screened a series of patients and controls and found strong

evidence for the abnormal presence of anti-GluR3 antibodies specifically in three of the four patients with Rasmussen's encephalitis. They went on to show that plasmapheresis had a transient but beneficial effect in one of the patients. These remarkable observations suggest a very novel mechanism for epileptogenesis: focal injury with disruption of the blood-brain barrier may, in some cases, provoke the induction of autoantibodies against CNS antigens that affect neuronal excitability. The abnormal excitability may lead to a repeated cycle in which focal seizure activity causes transient, seizure-induced disruption of the blood-brain barrier, further induction of autoantibodies, increased hyperexcitability and so on. It will certainly be interesting to determine whether such mechanisms play a causative role in Rasmussen's encephalitis and whether similar events occur in other epilepsy syndromes.

3. Epileptogenesis of generalized epilepsy

In contrast to the substantial advances in our understanding of potential mechanisms underlying focal epileptogenesis, far less headway has been made in identifying the mechanisms of epileptogenesis in most forms of generalized epilepsy. The slow progress is partly explained by the fact that the functional transition to an abnormal network in many generalized epilepsies appears to be developmentally regulated and our understanding of development in the mammalian CNS remains limited. This is compounded by the problem of trying to characterize the extremely complex phenotype in generalized epilepsy; i.e. the challenge of studying the vast neural network that participates in the initiation and propagation of generalized seizures. Nonetheless, recent experimental work in this field indicates that abnormalities of specific proteins, such as ion channels or receptors, are sufficient to produce the generalized seizure phenotype. Such findings provide an entry point into the problem of epileptogenesis of generalized epilepsy by focusing attention on the developmental regulation of the specific protein, or other components of the system that are potentially related to the protein. Examples of advances in this area are discussed below: absence epilepsy and mouse models of generalized epilepsies produced by transgenic technology.

3.1. Absence epilepsy

The electrophysiological hallmark of absence epilepsy is the bilaterally synchronous spike wave discharge (SWD), which arises from oscillatory rhythms generated by thalamocortical circuitry [41,42,109]. A series of studies over the past decade has drawn attention to the important role of GABA_B receptors and calcium channels in the pathogenesis of SWDs. GABA_B-mediated IPSPs appear to be generated by certain thalamic relay neurons and part of their effect is to elicit low-threshold Ca²⁺ potentials via T-type calcium channels [22,49]. These observations led to the hypothesis that GABA-mediated hyperpolarization could induce oscillatory behavior through reciprocal effects of T-currents and Ca²⁺-activated K⁺ currents [22]. Evidence supporting the role of GABA_B receptors in the pathogenesis of absence epilepsy includes the findings that GABA_B agonists such as baclofen exacerbate SWDs while GABA_B antagonists attenuate SWDs in various animal models of absence seizures [47,60]. Furthermore, the potential importance of T-currents in this process was demonstrated by Coulter et al., who showed that anticonvulsant drugs known to be clinically effective in treating absence seizures significantly decreased T-currents in dissociated thalamic neurons [20].

These various studies thus implicate GABA_B and T-channel function in the pathophysiology of absence epilepsy. Although it has yet to be shown that dysfunction of either or both of these systems is the underlying cause of absence seizures, it would certainly be interesting to determine whether developmental regulation of these proteins is related to the expression of absence seizures in humans or animal models. If so, this would provide a rationale for studying the hypothesis that the regulation of expression of GABA_B or T-channels in the thalamus underlies epileptogenesis in absence epilepsy.

3.2. Epilepsy models produced by transgenic technology

The rapid increase in the use of transgenic technology to study a variety of neurobiological questions has had something of an unexpected side-effect related to epilepsy research: some of the new mouse

lines have been found to have an epilepsy phenotype. These include mice with null mutations for the serotonin 5-HT_{2C} receptor [113] or the alpha subunit of calcium/calmodulin kinase II [73,100]. A number of mouse models of epilepsy with presumed single-gene mutations were previously recognized [83]. However, other than the EL mouse, which has recently been shown to have an abnormality of the ceruloplasmin gene [38], the genetic causes of the other naturally occurring epilepsy mice remain unknown. Thus, the new transgenic lines have suddenly introduced candidate genes that may be responsible for epileptogenesis. This work also raises the possibility that mutations in many different genes can give rise to a similar epilepsy phenotype. This may make the task of identifying the basis of epileptogenesis in certain human epilepsies extremely difficult.

4. Seizure-induced gene expression

4.1. Overview

In 1987, Morgan et al., showed that metrazol-induced seizures led to prompt, transient expression of c-fos, a protein involved in the control of gene transcription within the nucleus [79]. The fact that c-fos functions as a transcriptional activator suggested that seizure activity could lead to alterations in the expression of other genes and this has proved to be the case. As shown in Table 1, numerous studies using a variety of seizure models have now shown seizure-induced changes in the expression of additional transcriptional activators, neurotransmitters, neurotransmitter receptors, structural proteins, stress proteins, growth factors and other proteins. At this point, it is no longer surprising to think of seizures as having a rather dramatic effect on the pattern of gene expression within the CNS. In fact, one of the challenges in the field has been to determine the functional significance of a given change in gene or protein expression.

The discovery of seizure-induced gene expression provides an obvious potential link between the acute effects of seizures and the longer-term plastic changes that are thought to underlie certain forms of epilepto-

Table 1

Examples of genes or gene products shown to have changes in expression following seizure activity ^a

	References
<i>Transcription factors</i>	
c-fos	[17-19,23,27,40,45,57,79,81,85,99,115]
c-jun	[19,27,40,45,121]
KROX-20	[39,40,45]
zif-268	[19]
<i>Growth factors</i>	
nerve growth factor	[4,31,32,36,37]
brain-derived neurotrophic factor	[4,51,55,125]
neurotrophin-3	[92]
fibroblast growth factors	[11,32,90,91]
<i>Receptors / channels</i>	
GABA _A receptors	[33,54]
glutamate receptors	[33,122]
potassium channels	[117]
gap junctions	[82]
<i>Neuropeptides and related proteins</i>	
neuropeptide Y	[71]
prodynorphin	[26,78]
proenkephalin	[26,78,87]
oxytocin	[12]
vasopressin	[12]
thyrotropin-releasing hormone	[56]
corticotropin-releasing hormone	[10]
<i>Structural proteins</i>	
glial fibrillary acidic protein	[9,116]
GAP-43	[74]
alpha-tubulin	[89]
cyclophilin	[124]
<i>Stress proteins</i>	
72-kDa heat-shock protein	[65,69,118]
73-kDa heat-shock protein	[52,123]
<i>Others</i>	
calbindin-D28K	[53,65,67]
tyrosine hydroxylase	[10]
tissue plasminogen activator	[88]

^a Most studies of mRNA expression rely on Northern analysis or in situ hybridization and the basis of the altered levels of expression (e.g. increased transcription, modification of mRNA turnover) is not established. Also, in most cases a causal relationship between seizure activity and altered gene expression has not been shown. This is especially true for studies using convulsants such as kainate, where the effects of seizure activity versus neuronal injury are not readily distinguished.

genesis. The discussion below focuses on two particularly interesting examples: changes in the expression of ion channels and growth factors.

4.2. Changes in channel expression

Changes in ion channel expression following seizures are of obvious potential relevance to epileptogenesis, since they would be predicted to have an effect on membrane excitability. Previous studies

have provided evidence for functional and long-lasting changes in NMDA receptor expression in the hippocampus following kindling [72,77,80], although the exact molecular basis for this has not been determined [73]. In contrast, there are a few studies showing changes in expression of mRNA encoding channel subunits following seizures, but the functional effects of the altered level of transcripts remains unknown. For example, Tsaur et al. [117] have described short-term decreases in the expression of

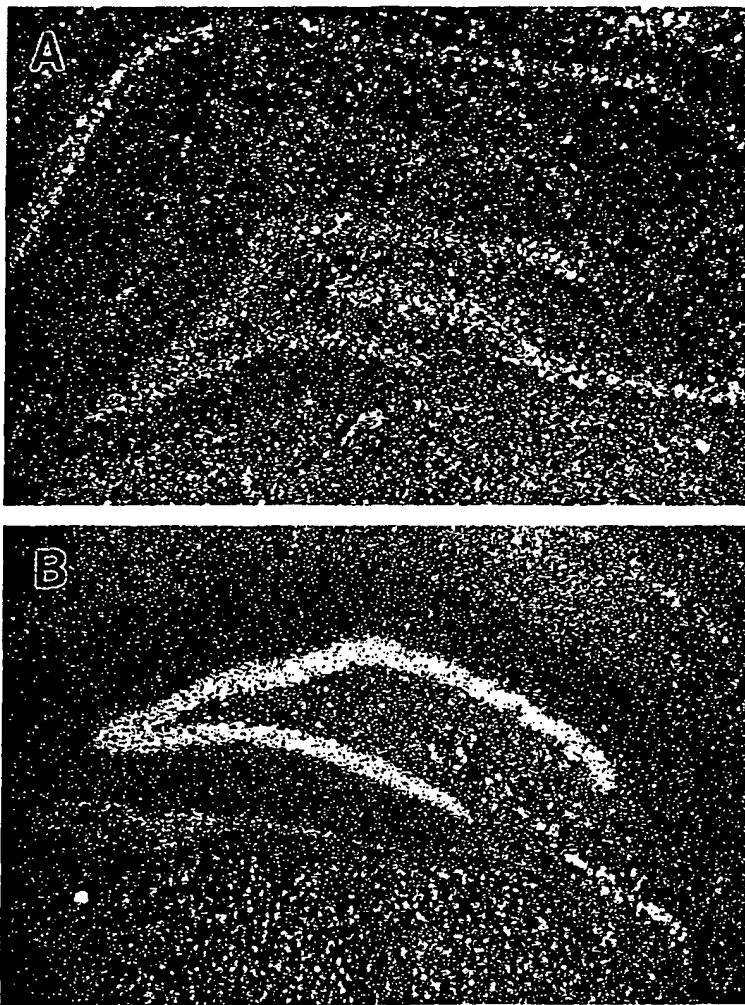


Fig. 4. Increased expression of nerve growth factor (NGF) mRNA in dentate granule cells 2.5 hours following kainate-induced seizures. Standard techniques of in situ hybridization with an NGF cRNA probe were used on hippocampal slices obtained from normal (A) or kainate-treated rats (B). The dark-field photomicrographs show a marked increase in signal from the NGF probe overlying the dentate granule cell layer. These results are very similar to those first reported by Gall et al. [36].

specific potassium channel subunit mRNAs in dentate granule cells following metrazol seizures, i.e. changes that might be expected to enhance granule cell excitability. Also, Friedman et al. [33] found a very interesting pattern of differential modulation of AMPA/kainate receptor subunit mRNA expression following kainate-induced seizures. There was a relative increase in GluR2 and GluR3 subunit expression in CA3/CA4 pyramidal cells, whereas GluR1 expression remained unchanged. Given the evidence that the presence of the GluR2 subunit in heteromultimeric receptor complexes reduces calcium permeability of the AMPA/kainate receptor [119], these findings raised the intriguing possibility that a shift in subunit composition might increase the susceptibility of the pyramidal cells to calcium-mediated glutamate excitotoxicity. Consistent with this idea, there was a decrease in GluR2 and GluR3 subunit expression in dentate granule neurons, which are relatively resistant to excitotoxic insults. Taken together, these various studies emphasize that seizures are likely to induce a wide variety of changes in channel and membrane receptor expression and these changes may alter both the excitability of neurons as well as their relative vulnerability to injury.

4.3. Changes in growth factor expression

A variety of growth factor genes have been shown to be up-regulated by seizure activity (see Table 1). Gall et al. [36] first showed that nerve growth factor (NGF) mRNA expression increased in specific hippocampal and cortical neurons within hours following an electrolytic hilar lesion (see Fig. 4). Subsequent studies have shown similar patterns of induction of NGF mRNA in other seizure or injury models [4,31,58]. Relatively acute changes in the expression of mRNAs encoding other growth factors such as brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and basic or acidic fibroblast growth factor (FGF) have also been documented [11,29,51,59,90,92,125]. Furthermore, there is evidence supporting the hypothesis that the upregulation of some growth factor mRNAs following seizures is related to increased expression of transcriptional activators. For example, the NGF gene promoter contains an AP-1 binding site that would make NGF-expression responsive to the presence of c-fos and

c-jun [44]. These findings suggest that, not surprisingly, seizure-induced gene expression occurs 'waves' of transcriptional changes in which one of events leads to the induction (or repression) of other factors that subsequently affect the expression of other genes.

Nonetheless, in trying to link the acute changes in growth factor expression to longer-term processes such as structural remodeling of the hippocampus following seizures, it is necessary to ask whether there are relatively prolonged changes (i.e. beyond the 12–24-hour period documented in most studies) in growth factor expression during these processes. To address this question, we recently determined whether changes in neurotrophin mRNA expression are associated with an increase in biologically active protein by studying hippocampal protein derived from rats sacrificed at various intervals between 12 hours and 2 months after kainate-induced seizures [68]. We found a marked increase in 'NGF-like' activity (assayed with an *in vitro* bioassay) that was maximal 7 days after seizures and remained sustained for at least 2 months. In a subsequent study, we investigated whether an antibody that was capable of blocking much of the extract activity *in vitro* would also block sprouting *in vivo*. Infusion of the anti-NGF antibody was found to partially block pilocarpine-induced cholinergic but not mossy fiber sprouting [66].

These experiments thus show that seizure-induced growth factor expression can occur over a relatively prolonged timecourse that parallels that of axonal reorganization. Furthermore, long-term expression of NGF appears to be functionally related to remodeling of cholinergic axons following seizures. Recent work by Repressa et al. [89] has provided further support for the concept that seizures induce prolonged expression of other molecules, such as structural proteins, predicted to be involved in network remodeling.

5. Conclusions

5.1. Implications for drug design and rational polypharmacy

Clinical observations alone make it clear that in some patients, an acute event such as head trauma or stroke initiates a change of network properties in

Table 2
Some key questions regarding epileptogenesis

1.	How reliable and valid are the current experimental models of epileptogenesis?
2.	Are pathological changes such as interneuron loss or axonal reorganization mechanistically linked to specific forms of epileptogenesis?
3.	How relevant are current models of epileptogenesis in the hippocampus to epileptogenesis in other brain regions?
4.	Are autoantibodies the primary defect in Rasmussen's encephalitis? Do similar mechanisms underlie other forms of epilepsy?
5.	How do seizure-induced changes in protein expression relate to the anatomical and physiological processes associated with epileptogenesis?

Table 3
Proposed studies of epileptogenesis

1.	Chronic, detailed electrophysiological recordings in animal models of epileptogenesis.
2.	Longitudinal anatomical and functional imaging studies of patients with risk factors for acquired epilepsy.
3.	Detailed analyses of the patterns of cell injury and network reorganization in surgical specimens and autopsy material from patients with epilepsy.
4.	Determination of whether the isolated loss of specific subpopulations of neurons (e.g. hilar mossy cells) is sufficient to cause chronic epilepsy in animal models.
5.	Identification of the specific effects of axonal reorganization on network excitability.
6.	Modulation of seizure-induced gene expression (e.g. using molecular pharmacology, transgenic systems, etc.) to identify the roles of specific gene products in epileptogenesis.

CNS that can ultimately lead to a seizure focus. Importantly, this transformation often occurs over a relatively long time period spanning from weeks to many years. Current therapy for epilepsy is primarily based upon the use of anticonvulsant compounds in patients who have already developed a seizure focus. An obvious implication of the work reviewed here is that an understanding of epileptogenesis could lead to therapeutic strategies designed to prevent or suppress the development of a seizure focus from the outset. For example, if selective hilar neuron loss proves to be a mechanistic basis for TLE, drugs which protect hilar neurons from the acute effects of head injury, or from subclinical excitation-induced injury, might prevent the critical loss of neurons that renders the dentate granule cells permanently hyperexcitable. Similarly, therapeutic strategies that antagonize maladaptive axonal reorganization, or enhance adaptive reorganization (or both) could also help prevent the emergence of a seizure focus.

5.2. Priorities for future research

If antiepileptogenic compounds are to become a viable form of therapy, then a major priority for

future research must be to further our understanding of the underlying mechanisms of epileptogenesis in various forms of epilepsy. Key questions and hypotheses regarding epileptogenesis are listed in Tables 2 and 3.

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References

- [1] Amaral, D., A golgi study of cell types in the hilar region of the hippocampus in the rat, *J. Comp. Neurol.*, 182 (1978) 851-914.
- [2] Annegers, J., Grabow, J., Groover, R., Laws, E., Elveback, L. and Kurland, L., Seizures after head trauma: A population study, *Neurology*, 30 (1980) 683-689.

- [3] Babb, R., Kupfer, W., Pretorius, J., Crandall, P. and Levesque, M., Synaptic reorganization by mossy fibers in human epileptic fascia dentata, *Neuroscience*, 42 (1991) 351-363.
- [4] Ballarín, M., Emfors, P., Lindfors, N. and Persson, H., Hippocampal damage and kainic acid injection induce a rapid increase in mRNA for BDNF and NGF in the rat brain, *Exp. Neurol.*, 114 (1991) 35-43.
- [5] Bear, J. and Lothman, E., An in vitro study of focal epileptogenesis in combined hippocampal-parahippocampal slices, *Epilepsy Res.*, 14 (1993) 183-193.
- [6] Bekenstein, J. and Lothman, E., Dormancy of inhibitory interneurons in a model of temporal lobe epilepsy, *Science*, 259 (1993) 97-100.
- [7] Ben-Ari, Y., Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy, *Neuroscience*, 14 (1985) 375-403.
- [8] Bertram, E., Lothman, E. and Lenn, N., The hippocampus in experimental chronic epilepsy, *Ann. Neurol.*, 27 (1990) 43-48.
- [9] Bonthius, D., Stringer, J., Lothman, E. and Steward, O., Spreading depression and reverberatory seizures induce the upregulation of mRNA for glial fibrillary acidic protein, *Brain Res.*, 645 (1994) 215-224.
- [10] Brady, L., Lynn, A., Glowa, J., Le, D. and Herkenham, M., Repeated electroconvulsive shock produces long-lasting increases in messenger RNA expression of corticotropin-releasing hormone and tyrosine hydroxylase in rat brain. Therapeutic implications, *J. Clin. Invest.*, 94 (1994) 1263-1268.
- [11] Bugra, K., Pollard, M., Charton, G., Moreau, J., Ben-Ari, Y. and Khrestchatsky, M., aFGF, bFGF and flg mRNAs show distinct patterns of induction in the hippocampus following kainate-induced seizures, *Eur. J. Neurosci.*, 6 (1994) 58-66.
- [12] Carter, D. and Murphy, D., Acute down-regulation of oxytocin and vasopressin mRNA levels following metrazole-induced seizure in the rat, *Neurosci. Lett.*, 160 (1993) 135-138.
- [13] Cavazos, J., Das, I. and Sutula, T., Neuronal loss induced in limbic pathways by kindling: Evidence for induction of hippocampal sclerosis by repeated brief seizures, *J. Neurosci.*, 14 (1994) 3106-3121.
- [14] Caveness, W., Meirowsky, A., Risk, B., Mohr, J., et al., The nature of posttraumatic epilepsy, *J. Neurosurg.*, 50 (1979) 545-553.
- [15] Cendes, F., Andermann, F., Gloor, P., Lopes-Cendes, I., Andermann, E., Melanson, D., Jones-Gotman, M., Robitaille, Y., Evans, A. and Peters, T., Atrophy of mesial structures in patients with temporal lobe epilepsy: Cause or consequence of repeated seizures?, *Ann. Neurol.*, 34 (1993) 795-801.
- [16] Claiborne, B., Amaral, D. and Cowan, W., A light and electron microscopic analysis of the mossy fibers in the rat dentate gyrus, *J. Comp. Neurol.*, 246 (1986) 435-458.
- [17] Clark, M., Post, R., Weiss, S., Cain, C. and Nakajima, T., Regional expression of c-fos mRNA in rat brain during evolution of amygdala kindled seizures, *Brain Res. Mol. Brain Res.*, 11 (1991) 56-61.
- [18] Clark, M., Post, R.M., Weiss, S.R. and Nakajima, T., Expression of c-fos mRNA in acute and kindled cocaine seizures in rats, *Brain Res.*, 582 (1992) 101-106.
- [19] Cole, A., Sawson, A., Saffen, D., Baraban, J. and Worley, P., Rapid rise in transcription factor mRNAs in rat brain after electroshock-induced seizures, *J. Neurochem.*, 55 (1990) 1920-1927.
- [20] Coulter, D., Huguenard, J. and Prince, D., Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons, *Ann. Neurol.*, 25 (1989) 582-593.
- [21] Cronin, J., Obenaus, A., Houser, C. and Dudek, F., Electrophysiology of dentate granule cells after kainate-induced synaptic reorganization of the mossy fibers, *Brain Res.*, 575 (1992) 305-310.
- [22] Crunelli, V. and Leresche, N., A role for GABA_B receptors in excitation and inhibition of thalamocortical cells, *Trends Neurosci.*, 14 (1991) 16-21.
- [23] Daval, J., Nakajima, T., Gleiter, C., Post, R. and Marangos, P., Mouse brain c-fos mRNA distribution following a single electroconvulsive shock, *J. Neurochem.*, 52 (1989) 1954-1957.
- [24] Davenport, C., Brown, W. and Babb, T., Sprouting of GABAergic and mossy fiber axons in dentate gyrus following intrahippocampal kainate in the rat, *Exp. Neurol.*, 109 (1990) 180-190.
- [25] de Lanerolle, N., Kim, J., Robbins, R. and Spencer, D., Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy, *Brain Res.*, 495 (1989) 387-395.
- [26] Douglass, J., Grimes, L., Shook, J., Lee, P. and Hong, J., Systemic administration of kainic acid differentially regulates the levels of prodynorphin and proenkephalin mRNA and peptides in the rat hippocampus, *Brain Res. Mol. Brain Res.*, 9 (1991) 79-86.
- [27] Dragunow, M., Yamada, N., Bilkey, D. and Lawlor, P., Induction of immediate-early gene proteins in dentate granule cells and somatostatin interneurons after hippocampal seizures, *Brain Res. Mol. Brain Res.*, 13 (1992) 119-126.
- [28] Du, F., Whetsell, W., Abou-Khalil, B., Blumenkopf, B., Lothman, E. and Schwarcz, R., Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy, *Epilepsy Res.*, 16 (1993) 223-233.
- [29] Dugich-Djordjevic, M., Tocco, G., Lapchak, P., Pasinetti, G., Najm, I., Baudry, M. and Hefti, F., Regionally specific and rapid increases in brain-derived neurotrophic factor messenger RNA in adult rat brain following seizures induced by systemic administration of kainic acid, *Neuroscience*, 47 (1992) 303-315.
- [30] Echlin, F. and Battista, A., Epileptiform seizures from chronic isolated cortex, *Arch. Neurol.*, 9 (1963) 154-170.
- [31] Ernfors, P., Bengzon, J., Kokaia, Z., Persson, H. and Lindvall, O., Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis, *Neuron*, 7 (1991) 165-176.

- [32] Follesa, P., Gale, K. and Mocchetti, I., Regional and temporal pattern of expression of nerve growth factor and basic fibroblast growth factor mRNA in rat brain following electroconvulsive shock, *Exp. Neurol.*, 127 (1994) 37-44.
- [33] Friedman, L., Pellegrini-Giampietro, D., Sperber, E., Ben-net, M., Moshe, S. and Zukin, R., Kainate-induced status epilepticus alters glutamate and GABA_A receptor gene expression in adult rat hippocampus: An in situ hybridization study, *J. Neurosci.*, 14 (1994) 2697-2707.
- [34] Frotscher, M. and Zimmer, J., Lesion-induced mossy fibers to the molecular layer of the rat fascia dentata: Identification of postsynaptic granule cells by the Golgi-EM technique, *J. Comp. Neurol.*, 215 (1983) 299-311.
- [35] Gale, K., Subcortical structures and pathways involved in convulsive seizure generation, *J. Clin. Neurophys.*, 9 (1992) 264-277.
- [36] Gall, C. and Isackson, P., Limbic seizures increase neuronal production of messenger RNA for nerve growth factor in adult rat forebrain, *Science*, 245 (1989) 758-761.
- [37] Gall, C., Murray, K. and Isackson, P., Kainic acid-induced seizures stimulate increased expression of nerve growth factor mRNA in rat hippocampus, *Brain Res. Mol. Brain Res.*, 9 (1991) 113-123.
- [38] Garey, C., Schwarzman, A., Rise, M. and Seyfried, T., Ceruloplasmin gene defect associated with epilepsy in EL mice, *Nature Genetics*, 6 (1994) 426-431.
- [39] Gass, P., Herdegen, T., Bravo, R. and Kiessling, M., High induction threshold for transcription factor KROX-20 in the rat brain: Partial co-expression with heat shock protein 70 following limbic seizures, *Brain Res. Mol. Brain Res.*, 23 (1994) 292-298.
- [40] Gass, P., Herdegen, T., Bravo, R. and Kiessling, M., Induction of immediate early gene encoded proteins in the rat hippocampus after bicuculline-induced seizures: Differential expression of KROX-24, FOS and JUN proteins, *Neuroscience*, 48 (1992) 315-24.
- [41] Gloor, P., Electrophysiology of generalized epilepsy. In: P. Schwarzkroin and H. Wheal (Eds.), *Electrophysiology of Epilepsy*, Academic Press, New York, 1984, pp. 107-136.
- [42] Gloor, P. and Fariello, R., Generalized epilepsy: Some of its cellular mechanisms differ from those of focal epilepsy, *Trends Neurosci.*, 11 (1988) 63-68.
- [43] Goddard, G., The development of epileptic seizures through brain stimulation at low intensity, *Nature*, 214 (1967) 1020-1021.
- [44] Hengerer, B., Lindholm, D., Heumann, R., Ruther, U. and Wagner, E., Lesion-induced increase in nerve growth factor mRNA is mediated by c-fos, *Proc. Natl. Acad. Sci. USA*, 87 (1990) 3899-3903.
- [45] Herdegen, T., Sandkuhler, J., Gass, P., Kiessling, M., Bravo, R. and Zimmermann, M., JUN, FOS, KROX and CREB transcription factor proteins in the rat cortex: Basal expression and induction by spreading depression and epileptic seizures, *J. Comp. Neurol.*, 333 (1993) 271-88.
- [46] Hoffman, S., Salin, P. and Prince, D., Chronic neocortical epileptogenesis in vitro, *J. Neurophys.*, 71 (1994) 1762-1773.
- [47] Hosford, D., Clark, S., Cao, Z., Wilson, W., Lin, F., Morrisett, R. and Huin, A., The role of GABA_B receptor activation in absence seizures of lethargic (lh/lh) mice, *Science*, 257 (1992) 392-401.
- [48] Houser, C., Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy, *Brain Res.*, 535 (1990) 195-204.
- [49] Huguenard, J. and Prince, D., A novel T-type current underlies prolonged Ca²⁺-dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus, *J. Neurosci.*, 12 (1992) 3804-3817.
- [50] Isokawa, M., Levesque, M., Babb, T. and Engel, J., Single mossy fiber axonal systems of human dentate granule cells studied in hippocampal slices from patients with temporal lobe epilepsy, *J. Neurosci.*, 13 (1993) 1511-1522.
- [51] Isackson, P., Huntsman, M., Murray, K. and Gall, C., BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: Temporal patterns of induction distinct from NGF, *Neuron*, 6 (1991) 937-948.
- [52] Kaneko, M., Abe, K., Kogure, K., Saito, H. and Matsuki, N., Correlation between electroconvulsive seizure and HSC70 mRNA induction in mice brain, *Neurosci. Lett.*, 157 (1993) 195-198.
- [53] Kohr, G., Lambert, C. and Mody, I., Calbindin-D28K (CaBP) levels and calcium currents in acutely dissociated epileptic neurons, *Exp. Brain Res.*, 85 (1991) 543-541.
- [54] Kokaia, M., Pratt, G., Elmer, E., Bengzon, J., Fritschy, J., Kokaia, Z., Lindvall, O. and Mohler, H., Biphasic differential changes of GABA_A receptor subunit mRNA levels in dentate gyrus granule cells following recurrent kindling-induced seizures, *Brain Res. Mol. Brain Res.*, 23 (1994) 323-332.
- [55] Kokaia, Z., Gido, G., Ringstedt, T., Bengzon, J., Kokaia, M., Siesjö, B., Persson, H. and Lindvall, O., Rapid increase of BDNF mRNA levels in cortical neurons following spreading depression: Regulation by glutamatergic mechanisms independent of seizure activity, *Brain Res. Mol. Brain Res.*, 19 (1993) 277-286.
- [56] Kubek, M., Knoblich, S., Sharif, N., Burt, D., Buterbaugh, G. and Fuson, K., Thyrotropin-releasing hormone gene expression and receptors are differentially modified in limbic foci by seizures, *Ann. Neurol.*, 33 (1993) 70-76.
- [57] Labiner, D., Butler, L., Cao, Z., Hosford, D., Shin, C. and McNamara, J., Induction of c-fos mRNA by kindled seizures: Complex relationship with neuronal burst firing, *J. Neurosci.*, 13 (1993) 744-751.
- [58] Lauterborn, J., Isackson, P. and Gall, C., Seizure-induced increases in NGF mRNA exhibit different time courses across forebrain regions and are biphasic in hippocampus, *Exp. Neurol.*, 125 (1994) 22-40.
- [59] Lindvall, O., Ernfors, P., Bengzon, J., Kokaia, Z., Smith, M., Siesjö, B. and Persson, H., Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor and neurotrophin 3 in the adult rat brain following cerebral ischemia and hypoglycemic coma, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 648-652.
- [60] Liu, Z., Vergnes, M., Depaulis, A. and Marescaux, C.,

- Involvement of intrathalamic GABA_B neurotransmission in the control of absence seizures in the rat. *Neuroscience*, 48 (1992) 87-93.
- [61] Livsey, C., Costa, E. and Vicini, S., Glutamate-activated currents in outside-out patches from spiny versus aspiny hilar neurons of rat hippocampal slices. *J. Neurosci.*, 13 (1993) 5324-5333.
- [62] Lothman, E. and Collins, R., Kainic acid induced limbic seizures: Metabolic, behavioral, electroencephalographic and neuropathological correlates. *Brain Res.*, 218 (1981) 299-318.
- [63] Lothman, E., Bertram, E., Kapur, J. and Stringer, J. Recurrent spontaneous hippocampal seizures in the rat as a chronic sequela to limbic status epilepticus. *Epilepsy Res.*, 6 (1990) 110-119.
- [64] Lowenstein, D., Basic concepts of molecular biology for the epileptologist. *Epilepsia*, 35 (Suppl. 1) (1994) S7-S19.
- [65] Lowenstein, D., Gwinn, R., Seren, S., Simon, R. and McIntosh, T., Increased expression of mRNA encoding calbindin-D28K, the glucose-regulated proteins, or the 72 kDa heat-shock protein in three models of acute CNS injury. *Brain Res. Mol. Brain Res.*, 22 (1994) 299-308.
- [66] Holtzman, D. and Lowenstein, D., Selective inhibition of axon outgrowth by antibodies to NGF in a model of temporal lobe epilepsy. *J. Neurosci.*, in press.
- [67] Lowenstein, D., Miles, M., Hatam, F. and McCabe, T., Up-regulation of calbindin-D28K mRNA in the rat hippocampus following focal stimulation of the perforant path. *Neuron*, 6 (1991) 627-633.
- [68] Lowenstein, D., Seren, M. and Longo, F., Prolonged increases in neurotrophic activity associated with kainate-induced hippocampal synaptic reorganization. *Neuroscience*, 56 (1993) 597-604.
- [69] Lowenstein, D., Simon, R. and Sharp, F., The pattern of 72-kDa heat shock protein-like immunoreactivity in the rat brain following flurothyl-induced status epilepticus. *Brain Res.*, 531 (1990) 173-182.
- [70] Lowenstein, D., Thomas, M., Smith, D. and McIntosh, T., Selective vulnerability of dentate hilar neurons following traumatic brain injury: A potential mechanistic link between head trauma and disorders of the hippocampus. *J. Neurosci.*, 12 (1992) 4846-4853.
- [71] Marksteiner, J., Ortler, M., Bellmann, R. and Sperk, G., Neuropeptide Y biosynthesis is markedly induced in mossy fibers during temporal lobe epilepsy of the rat. *Neurosci. Lett.*, 112 (1990) 143-148.
- [72] Martin, D., McNamara, J. and Nadler, J., Kindling enhances sensitivity of CA3 hippocampal pyramidal cells to NMDA. *J. Neurosci.*, 12 (1992) 1928-1935.
- [73] McNamara, J., Cellular and molecular basis of epilepsy. *J. Neurosci.*, 14 (1994) 3413-3425.
- [74] Meberg, P., Gall, C. and Routtenberg, A., Induction of FI/GAP-43 gene expression in hippocampal granule cells after seizures. *Brain Res. Mol. Brain Res.*, 17 (1993) 295-299.
- [75] Mello, L., Cavalheiro, E., Tan, A., Kupfer, W., Pretorius, J., Babb, T. and Finch, D., Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: Cell loss and mossy fiber sprouting. *Epilepsia*, 34 (1993) 985-995.
- [76] Mello, L., Cavalheiro, E., Tan, A., Pretorius, J., Babb, T. and Finch, D., Granule cell dispersion in relation to mossy fiber sprouting, hippocampal cell loss, silent period and seizure frequency in the pilocarpine model of epilepsy. *Epilepsy Res.*, (Suppl. 9) (1992) 51-59.
- [77] Mody, I. and Heinemann, U., NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. *Nature*, 326 (1987) 701-704.
- [78] Moneta, M. and Holt, V., Perforant path kindling induces differential alterations in the mRNA levels coding for prodynorphin and proenkephalin in the rat hippocampus. *Neurosci. Lett.*, 110 (1989) 273-278.
- [79] Morgan, J., Cohen, D., Hempstead, J. and Curran, T., Mapping patterns of c-fos expression in the central nervous system after seizure. *Science*, 237 (1987) 192-196.
- [80] Morrisett, R., Chow, C., Nadler, J. and McNamara, J., Biochemical evidence for enhanced sensitivity to N-methyl-D-aspartate in the hippocampal formation of kindled rats. *Brain Res.*, 496 (1989) 25-28.
- [81] Nakajima, T., Daval, J., Gleiter, C., Deckert, J., Post, R. and Marangos, P., c-fos mRNA expression following electrical-induced seizure and acute nociceptive stress in mouse brain. *Epilepsy Res.*, 4 (1989) 156-159.
- [82] Naus, C., Bechberger, J. and Paul, D., Gap junction gene expression in human seizure disorder. *Exp. Neurol.*, 111 (1991) 198-203.
- [83] Noebels, J., Mutational analysis of inherited epilepsies. In: A. Delgado-Escueta, A. Ward, D. Woodbury and R. Porter (Eds.), *Advances in Neurology*, 44th edition, Raven Press, New York, 1986, pp. 97-109.
- [84] Olney, J., Fuller, T. and deGubareff, T., Acute dendrotoxic changes in the hippocampus of kainate treated rats. *Brain Res.*, 76 (1979) 91-100.
- [85] Popovich, T., Represa, A., Crepel, V., Barbin, G., Beaudoin, M. and Ben-Ari, M., Effects of kainic acid-induced seizures and ischemia on c-fos-like proteins in rat brain. *Brain Res.*, 536 (1990) 183-194.
- [86] Prince, D. and Guo-Fang, T., Epileptogenesis in chronically injured cortex: In vitro studies. *J. Neurophys.*, 69 (1993) 1276-1291.
- [87] Przewlocka, B., Lason, W., Machelska, H. and Przewlocki, R., The effects of cocaine-induced seizures on the proenkephalin mRNA level in the mouse hippocampus: A possible involvement of the nitric oxide pathway. *Neurosci. Lett.*, 168 (1994) 81-84.
- [88] Qian, Z., Gilbert, M., Colicos, M., Kandel, E. and Kuhl, D., Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. *Nature*, 361 (1993) 453-457.
- [89] Represa, A., Pollard, H., Moreau, J., Ghilini, G., Krestchatsky, M. and Ben-Ari, Y., Mossy fiber sprouting in epileptic rats is associated with a transient increased expression of alpha-tubulin. *Neurosci. Lett.*, 156 (1993) 149-152.
- [90] Riva, M., Gale, K. and Mochetti, I., Basic fibroblast growth

- factor mRNA increases in specific brain regions following convulsive seizures, *Brain Res. Mol. Brain Res.*, 15 (1992) 311-318.
- [91] Riva, M., Donati, E., Tascadda, F., Zolli, M. and Racagni, G., Short- and long-term induction of basic fibroblast growth factor gene expression in rat central nervous system following kainate injection, *Neuroscience*, 59 (1994) 55-65.
- [92] Rocamora, N., Palacios, J. and Mengod, G., Limbic seizures induce a differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3, in the rat hippocampus, *Brain Res. Mol. Brain Res.*, 13 (1992) 27-33.
- [93] Rogers, S., Andrews, P., Gahring, L., Whisenand, T., Cauley, K., Crain, B., Hughes, T., Heinemann, S. and McNamara, J., Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis, *Science*, 265 (1994) 648-651.
- [94] Salazar, A., Jabbari, B., Vance, S., Grafman, J., Amin, D. and Dillon, J., Epilepsy after penetrating head injury. I. Clinical correlates, *Neurology*, 35 (1985) 1406-1414.
- [95] Salin, P., Parada, I., Hoffman, S., Tseng, G. and Prince, D., Axonal sprouting of adult rat neocortical pyramidal cells in chronic epileptogenic lesions, *Soc. Neurosci. Abstr.*, 19 (1993) 1031.
- [96] Scharfman, H., Characteristics of spontaneous and evoked EPSPs recorded from dentate spiny hilar cells in rat hippocampal slices, *J. Neurophys.*, 70 (1993) 742-757.
- [97] Scharfman, H. and Schwartzkroin, P., Protection of dentate hilar cells from prolonged stimulation by intracellular calcium chelation, *Science*, 246 (1989) 257-260.
- [98] Sharpless, S. and Halpern, L., The electrical excitability of chronically isolated cortex studied by means of permanently implanted electrodes, *Electroencephalogr. Clin. Neurophys.*, 14 (1962) 244-255.
- [99] Shin, C., McNamara, J., Morgan, J., Curran, T. and Cohen, D., Induction of c-fos mRNA expression by afterdischarge in the hippocampus of naive and kindled rats, *J. Neurochem.*, 55 (1990) 1050-1055.
- [100] Silva, A., Stevens, C., Tonegawa, S. and Wang, Y., Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice, *Science*, 257 (1992) 201-206.
- [101] Sloviter, R., 'Epileptic' brain damage in rats induced by sustained electrical stimulation of the perforant path. I. Acute electrophysiological and light microscopic studies, *Brain Res. Bull.*, 10 (1983) 675-697.
- [102] Sloviter, R., Permanently altered hippocampal structure, excitability and inhibition after experimental status epilepticus in the rat: The 'dormant basket cell' hypothesis and its possible relevance to temporal lobe epilepsy, *Hippocampus*, 1 (1991) 41-66.
- [103] Sloviter, R., Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats, *Neurosci. Lett.*, 137 (1992) 91-96.
- [104] Sloviter, R., The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy, *Ann. Neurol.*, 35 (1994) 640-654.
- [105] Sloviter, R. and Damiano, B., On the relationship between kainic acid-induced epileptiform activity and hippocampal neuronal damage, *Neuropharmacology*, 20 (1981) 1003-1011.
- [106] Sloviter, R. and Damiano, B., Sustained electrical stimulation of the perforant path duplicates kainate-induced electrophysiological effects and hippocampal damage in rats, *Neurosci. Lett.*, 24 (1981) 279-284.
- [107] Sloviter, R., Sollas, A., Barbaro, N. and Laxer, K., Calcium-binding protein (calbindin-D28K) and parvalbumin immunocytochemistry in the normal and epileptic hippocampus, *J. Comp. Neurol.*, 208 (1991) 381-196.
- [108] Spiller, A. and Racine, R., The effect of kindling beyond the 'stage 5' criterion on paired-pulse depression and hilar cell counts in the dentate gyrus, *Brain Res.*, 635 (1994) 139-147.
- [109] Steriade, M. and Llinas, R., The functional states of the thalamus and the associated neuronal interplay, *Physiol. Rev.*, 68 (1988) 649-742.
- [110] Sutula, T., Cascino, G., Cavazos, J., Parada, I. and Ramirez, L., Mossy fiber synaptic reorganization in the epileptic human temporal lobe, *Ann. Neurol.*, 26 (1989) 321-330.
- [111] Sutula, T., Xiao-Xian, H., Cavazos, J. and Scott, G., Synaptic reorganization in the hippocampus induced by abnormal functional activity, *Science*, 239 (1988) 1147-1150.
- [112] Tauck, D. and Nadler, J., Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats, *J. Neurosci.*, 5 (1985) 1016-1022.
- [113] Tecott, L., Sun, L., Akana, S., Strack, A., Lowenstein, D., Dallman, M. and Julius, D., Eating disorder and epilepsy in mice lacking functional 5HT_{2C} serotonin receptors, *Nature*, 374 (1995) 542-546.
- [114] Temkin, O., *The Falling Sickness: A History of Epilepsy from the Greeks to the Beginnings of Modern Neurology*, The Johns Hopkins Press, Baltimore, 1945.
- [115] Teskey, G., Atkinson, B. and Cain, D., Expression of the proto-oncogene c-fos following electrical kindling in the rat, *Brain Res. Mol. Brain Res.*, 11 (1991) 1-10.
- [116] Torre, E.R., Lothman, E. and Steward, O., Glial response to neuronal activity: GFAP-mRNA and protein levels are transiently increased in the hippocampus after seizures, *Brain Res.*, 631 (1993) 256-64.
- [117] Tsaor, M., Sheng, M., Lowenstein, D., Jan, Y. and Jan, L., Differential expression of K channel mRNAs in the rat brain and down regulation in the hippocampus following seizures, *Neuron*, 8 (1992) 1055-1067.
- [118] Vass, K., Berger, M., Nowak, T., Jr., Welch, W. and Lassmann, H., Induction of stress protein HSP70 in nerve cells after status epilepticus in the rat, *Neurosci. Lett.*, 100 (1989) 259-264.
- [119] Verdoon, T., Burnashev, N., Monyer, H., Seeburg, P. and Sakmann, B., Structural determinants of ion flow through recombinant glutamate receptor channels, *Science*, 252 (1991) 1715-1718.
- [120] Wilmore, L., Post-traumatic epilepsy: cellular mechanisms and implications for treatment, *Epilepsia*, (Suppl. 3) (1990) S67-S73.
- [121] Winston, S., Hayward, M., Nestler, E. and Duman, R.,

- Chronic electroconvulsive seizures down-regulate expression of the immediate-early genes c-fos and c-jun in rat cerebral cortex. *J. Neurochem.*, 54 (1990) 1920-1925.
- [122] Wong, M., Smith, M., Licinio, J., Doi, S., Weiss, S., Post, R. and Gold, P., Differential effects of kindled and electrically induced seizures on a glutamate receptor (GluR1) gene expression. *Epilepsy Res.*, 14 (1993) 221-227.
- [123] Wong, M., Weiss, S., Gold, P.W., Doi, S., Banerjee, S., Licinio, J., Lad, R., Post, R. and Smith, M., Induction of constitutive heat shock protein 73 mRNA in the dentate gyrus by seizures. *Brain Res. Mol. Brain Res.*, 13 (1992) 19-25.
- [124] Yount, G., Gall, C. and White, J., Limbic seizures increase cyclophilin mRNA levels in rat hippocampus. *Brain Res. Mol. Brain Res.*, 14 (1992) 139-142.
- [125] Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H. and Lindholm, D., Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO J.*, 9 (1990) 3545-3550.

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